

Kim et al., <http://www.jem.org/cgi/content/full/jem.20070109/DC1>

SUPPLEMENTAL MATERIALS AND METHODS

The primer sequence for methylation analysis. The FoxP3 promoter region was PCR amplified using the following nested primers: outer primer forward, 5'-TTTGATTTGATTATTTTTT-3'; outer primer reverse, 5'-ATACTAATAAACCTAACACCCACC-3'; inner primer forward, 5'-TATATTTTAGATGATTGTAAAGGGTAAA-3'; and inner primer reverse, 5'-ATCAACCTAACTTAAAAACTACCACAT-3'. The FoxP3 CpG island region was PCR amplified using the following nested primers: outer primer forward, 5'-TATTTTTGGGTTTGGGATATTA -3'; outer primer reverse, 5'-AACCAACCAACTCCTACACTATCTAT -3'; inner primer forward, 5'-TTTGGGTTTTTG-GTATTAAGA -3'; and inner primer reverse, 5'-TTAACCAAATTTCTACCATTAAC -3'.

The primer sequence for restriction enzyme accessibility assay. The primers and probes used for the PCR were: 5'-CTAATACGACTCACTATAGGGC-3' (T7 primer); 5'-TCGAGCGGCCGCCGGCAGGT-3' (nested primer); 5'-CCTTCTGCTCCAACCTCAGTATT-3' (promoter primer); 5'-(6-FAM)-TGGTGGTGATCATATGCAT-GCTTGTAAAG-(TAMRA-6-FAM)-3' (promoter probe); 5'-TGTGACAACAGGGCCCAGAT-3' (CNS3 primer); 5'-(6-FAM)-AGGAAAACATATTCTATGTCCCAGAAACACCTCCA-(TAMRA-6-FAM)-3' (CNS3 probe); 5'-AGAGGGAAATCGTGCAC-3' (actin primer); 5'-(6-FAM)-CACTGCCGCATCCTTCCTCCC-(TAMRA-6-FAM)-3' (actin probe).

The primer sequence for chromatin immunoprecipitation assay. The sequences of the primers and Taqman probe for the FoxP3 promoter were as follows: 5'-GCCAAGCCTGGCAACAT-3', 5'-CCTTCTGCTCCAACCTCAG-TATTT-3', and 5'-(6-FAM)-TGGTGGTGATCATATGCATGCTTGTAAAG-(TAMRA-6-FAM)-3', respectively. The sequences of the primers and Taqman probe for the intronic CpG island were 5'-TGTGACAACAGGGCCCAGAT-3', 5'-GCCTCCTGTTGACTGTTCTT-3', and 5'-(6-FAM)-AGGAAAACATATTCTATGTCCCAGAAA-CACCTCCA-(TAMRA-6-FAM)-3', respectively.